A Research Proposal

on

Development and Characterization of Bipolymer based Nanoparticulate Carrier System as Vaccine Adjuvant for Effective Immunization

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Submitted by:
Ajay Verma

Supervised by:
Dr.Arun Gupta
Cosupervised by:
Dr. Amit Mittal

FACULTY OF APPLIED MEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY
PUNJAB

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1. INTRODUCTION

1.1 Vaccines

The term "vaccine" derives from Edward Jenner's 1796 use of the term "cow pox" (Latin "variolæ vaccinæ", adapted from the Latin "vaccīn-us", from "vacca" cow), which, when administered to humans, provided them protection against small pox.

A **vaccine** is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters (Cohen et al., 2006).

Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g. vaccines against cancer (Ryan et al.,2001).

1.2 Adjuvants

An **adjuvant** is an agent that may stimulate the immune system and increase the response to a vaccine, (Elamanchili and Diwan,2002) without having any specific antigenic effect in itself . The word “adjuvant” comes from the Latin word adiuvare, meaning to help or aid. "An immunologic adjuvant is defined as any substance that acts to accelerate, prolong, or enhance antigen-specific immune responses when used in combination with specific vaccine antigens.

1.3 Adjuvants and vaccine

Several factors have been largely responsible for the inability of vaccines to protect (Holmgren et al., 2003) against infectious diseases:

1. One of the most significant factors includes the unavailability of vaccines against intracellular pathogens, or infected or altered cells, such as malaria and HIV, which rely on cell-mediated immunity.
2. Second, progress in the field of adjuvants for use with human vaccines has been inadequate, and the existing adjuvants, mainly aluminum compounds, often prohibiting optimization of the magnitude and direction of the immune response (i.e., the role of the adjuvant). Aluminum compounds (often termed alum) are well known for their inability to induce cell-mediated immunity. Other adjuvants such as calcium compounds and MF59 has limited potential for cell-mediated immunity.
3. Third, high dropout rates for receiving booster vaccine doses have left significant fractions of people in developing countries not fully immunized, often due to poor or limited access to medical care, and lack of education regarding the importance of booster vaccination.
4. Finally, incompletely immunized women (mostly in developing countries) cannot pass immunity to neonates, leaving newborns susceptible to infections, e.g., umbilical cord infections.

Whereas aluminum compounds have been used with a large number of antigens, these adjuvants are not suitable for all antigens, (Elamanchili et al., 2004) some limitations are :

a) Variable or poor adsorption of some antigens
b) Difficulty to lyophilize
c) The general requirement for booster doses
d) Inability to elicit cytotoxic T-cell (CTL) responses
e) Inability to elicit mucosal IgA antibody responses
f) Rare occurrence of hypersensitivity reactions in some subjects.

1.4 Polymer : As Adjuvant

a. Aliphatic polymers

Poly(D,L-lactic acid) (PLA) and poly(D,L-lactic-co-glycolicacid) (PLGA) are synthetic biodegradable polymers derived from lactic acid[5]. Their structures consist of L-, D- and D,L-lactic acid in which the D,L- polymers are amorphous and more rapidly degradable than their L- or D,L-forms. PLA and PLGA undergo hydrolysis in the body to produce their monomers. Polylactides, such as PLA, have been used successfully in food packaging applications due to their easy degradation, and packaging performance characteristics. Since PLGA is biocompatible, it has been used for implanted medical devices such as sutures, drug delivery and micro- or nanosphere controlled release systems. Another successful application of PLA and its co-copolymer PLGA is in producing dispersions under micro-order (Cun et al.,2011).

Polymer-based delivery systems may provide an increased physical and chemical stability of nanodispersed formulations containing active compounds over a period of storage time. Some studies have proposed the preparation of PLA and PLGA micro- and nanodispersions using solvent displacement methods, in which acetone and/or ethanol are generally used. PLGA is a polyester composed of one or more of three different hydroxy acid monomers, d-lactic,l-lactic, and/or glycolic acids. In general, the polymer, can be made to be highly crystalline [e.g., poly(l-lactic acid)], or completely amorphous[e.g., poly(d,l-lactic-co-glycolic acid), can be processed into most any shape and size (down to b200nm), and can encapsulate molecules of virtually any size. PLGA microspheres and other injectable implants have a long safety record and are used in at least 12 different marketed products from 10 different companies worldwide.

These controlled release products are capable of controlling the release of peptides and proteins slowly and continuously from 1 to 4 months. Early studies with PLGA, many of which were focused on depot formulations for LHRH analogs established several important principles, and indeed overcame several issues relevant to PLGA microparticles for antigen delivery (e.g., low protein entrapment during encapsulation, high initial burst and microparticle dispersibility to allow injection). For example, Hutchinson described how release kinetics of acid-stable peptides in PLGA films and microparticles could be made to be discontinuous or continuous by adjusting the polymer molecular weight and peptide loading; that is, medium to high molecular

weight and low protein loading favored discontinuous release and low molecular weight and high protein loading favored continuous release. Similarly, the lag time before the fast peptide release period in the discontinuous formulations, which resembled the time interval before booster dose of antigen, could be controlled by adjusting the polymer lactic/ glycolic acid ratio, with high lactic acid content favoring long lag times before polymer mass loss and peptide release. Such early studies were critical in demonstrating the versatility of PLGA to adjust release kinetics.

However, the fact that most peptides used in these studies were relatively stable in acidic media (e.g., leuprolide, groserelin and octreotide). PLGA produces significant acid upon polyester hydrolysis foreshadowed the later difficulties with stability of PLGA-encapsulated antigens, many of which are acid-labile. In addition to the depot effect, smaller PLGA microparticles (e.g., 10 Am) were demonstrated to have adjuvant activity via their uptake by macrophages and dendritic cells (DCs), and their localization in lymph nodes and to induce CTL responses . Despite the excellent biocompatibility of PLGA, the mild inflammatory response produced by PLGA microspheres has also been hypothesized as being involved in their adjuvant characteristics. Most significant were reports of long-lasting antibody responses, many neutralizing above protective levels, in numerous animal models following a single dose of PLGA microparticle encapsulated antigens including displays of immunological memory after 1 year of immunization and protection against challenge (Raghuvanshi et al., 2002).

b. Gelatin

Gelatin nanoparticles have been chosen as promising drug delivery system candidate. Typically, this natural biopolymer is present in other fields of our daily life. It gives gummi bears consistency and without gelatin containing icing, ingredients such as fruits would not stick to a cake. Consequently, the foodstuff industry is the major purchaser of the tonnages of gelatin that are produced every year. However, the amount of gelatin being applied in pharmaceutical industry is not negligible, as far as capsules and ointments are concerned.But also for current research in fields of delivery vehicles for the controlled release of biomolecules such as proteins and nucleotides, gelatin has generated increased interest (Young *et al.* 2005). While gelatin and the delivery systems based on this polymer are biocompatible and biodegradable without toxic

degradation products (Tabata & Ikada 1998 ; Young *et al*. 2005), they are furthermore known for high physiological tolerance and low immunogenicity since decades. However, rare ethnologically caused cases of hypersensitivity reactions in the Japanese population have been described in literature (Kelso 1999; Saito *et al.* 2005). But the basically beneficial properties of gelatin contributed to its proven record of safety which is also documented by the classification as "Generally Recognized as Safe" (GRAS) excipient by the US Food and Drug Administration (FDA). So, gelatin derivatives are even constituent of intravenously administered applications as plasma expanders (e.g. Gelafundin™, Gelafusal™) and used as sealant for vascular prostheses (Kuijpers *et al.* 2000). The natural source of gelatin are animals. It is obtained by mainly acidic or alkaline, but also thermal or enzymatic degradation of the structural protein collagen. Collagen represents 30% of all vertebrate body protein. More than 90% of the extracellular protein in the tendon and bone and more than 50% protein in the skin consist of collagen (Friess 1998). The characteristic molecular feature of collagen being responsible for its high stability is the unique triple-helix structure consisting of three polypeptide α-chains. Among the 27 collagen types that have been isolated so far (Brinckmann *et al.* 2005), only collagen type I (skin, tendon, bone), type II (hyaline vessels) and type III are utilized for the production of gelatin. According to origin and pretreatment of the utilized collagen, two major types of gelatin are commercially produced. Gelatin type A (**a**cid) is obtained from porcine skin with acidic pre-treatment prior to the extraction process. The second prevalent gelatin species, type B (**b**asic), is extracted from ossein and cut hide split from bovine origin. Thereby, an alkaline process, also known as "liming" is applied. During this extraction also the amide groups of asparagine and glutamine are targeted and hydrolyzed into carboxyl groups, thus converting many of the residues to aspartate and glutamate (Tabata & Ikada 1998; Young *et al.* 2005). Consequently, the electrostatic nature is affected, in contrast to collagen and gelatin type A having an isoelectric point (IEP) of pH 9.0, the higher number of carboxyl groups per molecule reduces the IEP to pH 5.0. Aside from the two major gelatin types, mixtures of both, resulting in specific intermediate IEPs and cold water fish gelatin do exist. Latterly, FibroGen (South San Francisco, CA, USA) offers synthetic gelatin produced by recombinant DNA technology via a yeast system.

1.5 Nanoparticles

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides, proteins and oligonucleotides (Catarina et al., 2006). The development of suitable carrier systems remains a major challenge for the pharmaceutical scientist, especially after oral/nasal application/ vaccination since bioavailability is limited by the mucosal barriers of the gastrointestinal tract/nasal route and degradation by digestive enzymes.

Polymeric nanoparticles (NP), defined as solid particles with a size in the range of 10-1000 nm, allow encapsulation of the drugs inside a polymeric matrix, protecting them against enzymatic and hydrolytic degradation. Not only enhancement of oral peptide bioavailability, but also the concept of mucosal vaccination has become more prominent in recent years (Kaumaresh et al., 2001). Oral and nasal dosage forms improve patient compliance and facilitate frequent boosting, necessary to achieve a protective immune response. Epithelial surfaces are the port of entry for many viral or bacterial pathogens, and mucosal immunity can be regarded as an important protective barrier.

Size is the parameter that has been identified as critical for the transport across the nasal mucosa. In this regard, there is a consensus on the fact that the particle transport is more important for nanoparticles than for microparticles. Accordingly, some authors observed that the size of the particles may influence the immune responses elicited following nasal administration of antigens encapsulated into these polymeric systems. For example, Somavarapu et al., and more recently, Jung et al. showed that the immune response to encapsulated antigens administered intranasally was significantly greater for nanoparticles than for microparticles.

Biodegradable nanoparticles used as controlled release (CR) systems are important in pharmaceutics. The basic idea to try to accomplish in drug delivery applications is that biodegradable nanoparticles degrade within the body as a result of natural biological processes, thereby eliminating the need to remove the delivery system after its function is over. Ability of polysaccharides to form a network structure (gel), even at low concentrations has been well known. This property to form a three-dimensional-network structure (gelation) offers an effective means of increasing the chemical stability and mechanical properties of the polymer ((Leo et al., 1997).

Protein-loaded nanoparticles have been extensively formulated for decades in the aim to formulate pharmaceutically applicable nanoparticles containing bioactive proteins. Most bioactive proteins of their native forms have a short half life in serum, less than 24 h, encapsulating proteins within nanoparticles has been an attractive way of overcoming major disadvantages of protein based pharmaceutics. Furthermore, proteins are released out in a controllable manner rather than a simple diffusion when biodegradable polymers are employed to formulate polymeric nanoparticles encapsulating protein drugs. In attempts to control the release rate of bioactive proteins encapsulated within polymeric nanoparticles, molecular weight and hydrophilicity of the polymer was widely modified to control degradation rates of polymeric nanoparticles. However, those approaches were simply dependent on degradation rates of polymers, therefore, could not reflect *in-vivo* physiological changes including temperatures, glucose levels, and pH, where pharmaceutical drug should be released according to micro-environmental changes *in-vivo*.

The traditional role of polymers in colloidal drug delivery systems has been that of an inert excipient. Since these materials are absorbed into the organism additional requirements need to be considered, such as biocompatibility and biodegradability. Moreover, physicochemical properties of polymers influence the formation of NP regarding size and encapsulation efficiency. Therefore, new biomaterials combining all these considerations might be of general interest for the design of nanoparticulate carrier systems for mucosal peptide delivery.

1.6 PLGA Coated Gelatin Nanoparticles

For effective mucosal immunization PLGA coated gelatin nanoparticles can be a useful approach as by this combination lot of problems assocaiated with the protein delivery can be overcome .PLGA coated gelatin nanoparticles can be formed by double emulsification solvent evaporation method (Coester et al., 2006) with slight modification. Gelatin nanoparticles required to be formed first followed by coating of nanoparticles by PLGA using double emulsification method.

Advantages

- Provide prolonged drug release due to PLGA
- This system also demonstrates the capability of preventing the denaturation of protein drugs.

- The hydrophilic-hydrophobic composite provides a high degree of biocompatibility.
- The gelatin nanoparticle-PLGA microsphere system is a burst release inhibitor.
- Increased T-cell mediated response.

1.7 TT (Tetanus Toxoid) as model antigen

Tetanus Toxoid is a tetanus toxin inactivated with formaldehyde to be immunogenic but not pathogenic. The vaccine can be formulated as simple or adsorbed Tetanus vaccine, combined Tetanus and Killed Polio vaccine, or the other. Because of its high immunogenicity Tetanus Toxoid (TT) can be used as a model antigen, which can help to identify the humoral as well as cell mediated immunological effects .

1.8 Nasal Delivery : The Route

Conventionally, the nasal route of delivery has been used for delivery of drugs for treatment of local diseases such as nasal allergy, nasal congestion and nasal infections. Recent years have shown that the nasal route can be with priority for the systemic delivery of drugs such as small molecular weight polar drugs, peptides and proteins that are not easily administered via other routes. Mucosal epithelia comprise an extensive vulnerable barrier which is reinforced by numerous innate defence mechanisms cooperating intimately with adaptive immunity. Local generation of secretory IgA (sIgA) constitutes the largest humoral immune system of the body. Secretory antibodies function both by performing antigen exclusion at mucosal surfaces by virus and endotoxin neutralization within epithelial cells without causing tissue damage (Cristoph et al.,2011).

Advantages of nasal route over other routes

- It has a good blood supply, a large surface area, nasal associated lymphoid tissue (NALT) and its local draining lymph nodes lying under the respiratory epithelium.
- Nasal immunisation not only presents antigen at a mucosal surface, where it may elicit a local immune response (sIgA), but the antigen may pass into the circulatory system and stimulate systemic IgG response.

- Local immune response allows first line defence against numerous respiratory bacterial and viral diseases, along with dissemination of the immune response to distant mucosal sites via the common mucosal immune system (CMIS).
- Although the oral route is much preferred, in comparison to oral immunization, IN immunization generally requires much lower doses of antigen, which has important implications for many recombinant antigens, which are often costly.
- In contrast to immunization of the genital tract, IN immunization is more convenient and acceptable, and has been shown to induce much more potent local and systemic responses.
- Finally, compared to rectal immunization the nasal route is more readily accessible and culturally more acceptable.

1.8.1. The mucosal surface

The nasal mucosa is lined with extensive pseudostratified columnar epithelium and includes ciliated cells and mucous-secreting goblet cells. Cilia are present at the apical surface, and mucous resides within the apices of the flask-shaped goblet cells (Illum, 2001).

Cilia are long (4–6 mm) thin projections, which are mobile and beat with a frequency of 1000 strokes per min. The beat of each cilium consists of a rapid forward movement. In this way the mucus layer is propelled in a direction from the anterior towards the posterior part of the nasal cavity. The mucus flow rate is in order of 5 mm/min and hence the mucus layer is renewed every 15–20 min.

The epithelium rests on a lamina propria of loose connective tissue, containing mucous glands. Whilst tight junctions seal intercellular pathways, agents that disrupt these may facilitate the transport of molecules through to the underlying connective tissue. The pseudostratified columnar epithelium is found throughout the nasal cavity, trachea, bronchi and bronchioles. It is generally believed that the mucosal immune system has evolved alongside, but separate from, the general systemic immune system, and as a major consequence of this dichotomy, only immune responses initiated in mucosal inductive sites can result in effective immunity in mucosal tissues themselves.

1.8.2 . Barriers to nasal absorption

Lipophilic drugs are generally well absorbed from the nasal cavity with the pharmacokinetic profiles often identical to those obtained after an intravenous injection and bioavailabilities approaching 100%. However, despite the large surface area of the nasal cavity and the extensive blood supply, the permeability of the nasal mucosa is normally low for polar molecules, to include low molecular weight drugs and especially large molecular weight peptides and proteins.

Thus, the most important factor limiting the nasal absorption of polar drugs and especially large molecular weight polar drugs, such as peptides and proteins, is the low membrane permeability. Drugs can cross the epithelial cell membrane either by

- The transcellular route uses simple concentration gradients, receptor mediated transport
- Vesicular transport mechanisms.
- The paracellular route through the tight junctions between the cells.

Polar drugs with molecular weights below 1000 Da will generally pass the membrane using the latter route, since, although tight junctions are dynamic structures and can open and close to a certain degree, when needed, the mean size of the channels is in the order of less than 10 Å and the transport of larger molecules is considerably more limited. Larger peptides and proteins have been shown to be able to pass the nasal membrane using an endocytotic transport process but only in low amounts.

Another factor of importance for low membrane transport is the general rapid clearance of the administered formulation from the nasal cavity due to the muco illiary clearance mechanism. It has been shown that for both liquid and powder formulations, that are not mucoadhesive, the half life of clearance is in the order of 15–20 min.

Another contributing (but normally considered less important) factor to the low transport of especially peptides and proteins across the nasal membrane is the possibility of an enzymatic degradation of the molecule either within the lumen of the nasal cavity or during passage across the epithelial barrier.

1.8.3. Secretory immunity

The secretory antibody, basis for immune exclusion, is locally provided by the mucosae and associated exocrine glands which harbor most of the body's activated B cells—terminally differentiated to Ig-producing plasmablasts and plasma cells (PCs).

Secretory IgA (sIgA) antibodies are remarkably stable hybrid molecules, mainly consisting of PC-derived IgA dimers with one (or more) 'joining' (J) chain(s) and an epithelial portion called bound secretory component (SC) which is disulfide linked to one of the IgA subunits. Most mucosal PCs (70–90%) do normally produce dimers and some trimers of IgA (collectively called polymeric IgA, pIgA) which, together with J chain containing pentameric IgM, are exported by secretory epithelial cells to provide sIgA and secretory IgM (sIgM) antibodies.

1.8.4. Stimulation of mucosal immunity

1.8.4.a Immune-inductive lymphoepithelial tissue

The various secretory effector sites receive their activated B cells from inductive mucosa associated lymphoid tissue **(MALT),** organized lymphoid structures, that sample antigens directly from the epithelial surface. Although gut-associated lymphoid tissue **(GALT)** – including Peyer's patches in the distal ileum, the appendix and numerous isolated lymphoid follicles – constitutes the major part of MALT, induction of mucosal immune responses can take place also in the palatine tonsils and other lymphoepithelial structures of Waldeyer's pharyngeal ring, including nasopharynx associated lymphoid tissue **(NALT).** Naıve B and T cells enter MALT (and lymph nodes) via high endothelial venules (HEVs). After being primed to become memory/effector B and T cells, they migrate from MALT and local lymph nodes.The endothelial cells exert a local gatekeeper function for mucosal immunity.

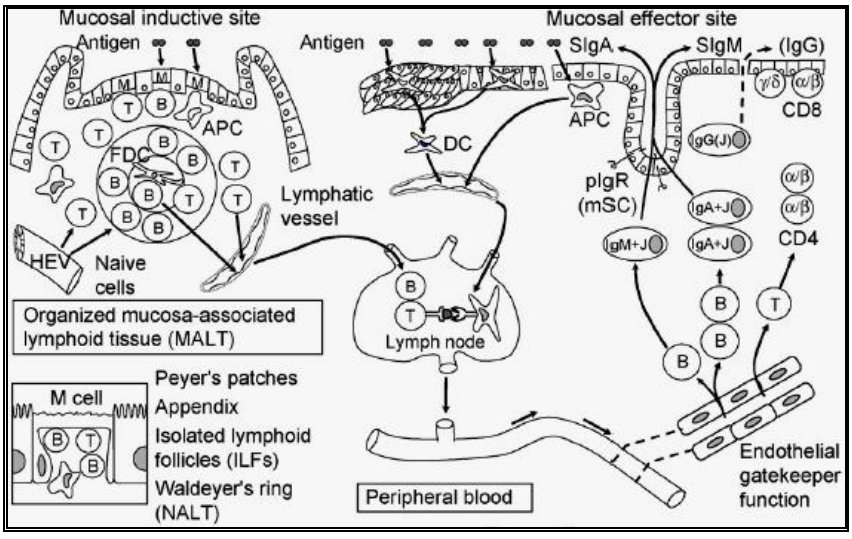

Fig. 1. Depiction of the human mucosal immune system.

1.8.4.b. Priming of mucosal B and T cells

Naive B and T cells arrive in MALT via high endothelial venules like in other secondary lymphoid tissue. Antigens are presented to the naive T cells by APCs after intracellular processing to immunogenic peptides. In addition, luminal peptides may be taken up and presented by B lymphocytes and epithelial cells to subsets of intra- and subepithelial T lymphocytes. Interestingly, MHC class II-expressing naive and memory B cells aggregate together with T cells in the M-cell pockets, which thus may represent the first contact site between immune cells and luminal antigens.

The activated B cells probably perform important antigen-presenting functions in this compartment, perhaps promoting antibody diversification and immunological memory.

1.8.4. Nasal delivery of vaccines

Many diseases, such as measles, pertussis, meningitis and influenza are associated with the entry of pathogenic microorganisms across the respiratory mucosal surfaces and are hence good candidates for nasal vaccines. The main reasons are given below. It is well established that nasally administered vaccines, especially if based on attenuated live cells or adjuvanted by means of an immunostimulator or a delivery system, can induce both mucosal and systemic (i.e. humoral and cell-mediated) immune responses. The nasal passages are rich in lymphoid tissues and the target site for nasal administered vaccines in man is considered to be nasal-associated lymphoid tissue (NALT) which is situated mainly in the pharynx as a ring of lymphoid tissue and named Waldeyer's ring.

1.8.4.1 Antimicrobial effects of sIgA antibodies

Provide efficient microbial agglutination and virus neutralization

- carry out anti-inflammatory extra- and intracellular immune exclusion by inhibiting epithelial adherence and invasion
- Exhibit extensive cross-reactive ('innate-like') activity and provide cross-immunity and herd protection
- sIgA (particularly the sIgA2 isotype) is quite resistant against proteolysis (bound SC stabilizes both isotypes)
- sIgA exhibits mucophilic and lectin-binding properties (via bound SC in both isotypes and mannose in sIgA2)

1.8.4.2 Main reasons for selecting the nasal route for vaccine delivery

The nasal mucosa is the first site of contact with inhaled antigens (Cristoph et al., 2011)

- The nasal passages are rich in lymphoid tissue (Nasal Associated Lymphoid Tissue-NALT).NALT is known as Waldeyer's ring in humans
    - Adenoid or nasopharyngeal tonsils
    - Bilateral lymphoid bands
    - Bilateral tubal and facial or palatine tonsils
    - Bilateral lingual tonsils
- Creation of both mucosal (sIgA) and systemic (IgG) immune responses
- Low cost, patient friendly, non-injectable, safe

Locally produced secretory IgA is considered to be among the most important protective humoral immune factors. This antibody constitutes over 80% of all antibodies produced in mucosa associated tissues. Soluble antigens penetrate the whole nasal epithelium and reach the superficial cervical lymph nodes: these antigens preferentially induce a systemic immune response (IgG).

Particulate antigens are more easily removed by the cilia in the NALT. However, viable pathogens are able to circumvent this method of excretion, and are taken up by the M-cells, which are morphologically and functionally comparable to microfold cells in the gut. After antigen has been transported to the NALT underneath epithelium, mucosal immune reactions (IgA) are elicited. Mucosal vaccines approved for the human use are depicted below.

1.8.4.3 Mucosal vaccines approved for human use

Oral live attenuated (Sabin) polio vaccine

- Oral killed whole-cell/B-subunit cholera vaccine
- Oral live attenuated cholera vaccineOral live attenuated typhoid vaccine
- Oral live attenuated adenovirus vaccine(restricted to military personnel)
- New oral live attenuated rotavirus vaccines: Rotarix and RotaTeq
- Nasal enterotoxin-adjuvanted inactivated influenza vaccine (Nasalflu);
- Nasal live attenuated influenza vaccine (FluMist)

2. LITERATURE REVIEW

Alpar et al.,2005 formulated biodegradable nanoparticles for mucosal antigen and DNA delivery. The formulation showed the potential of uptake of vaccine formulations by the primary olfactory nerves in the nasal cavity, effective delivery to the lung, strategies to maximise the immunopotentiation of candidate vaccine formulations, as well as the evaluation of animal models and interpretation of engendered immune responses in terms of antigen-specific antibody production. Experimental data demonstrated the potential of muco- and bioadhesive agents in combination with liposomes for intranasal (i.n.) delivery of tetanus toxoid in mice.

Brandtzaeg et al., 2006 studied on induction of secretory immunity and memory at mucosal surfaces. Identified that the intranasal route of vaccine application targeting nasopharynx-associated lymphoid tissue may be more advantageous for certain infections, but only if successful stimulation is achieved without the use of toxic adjuvants that might reach the central nervous system. The degree of protection obtained after mucosal vaccination ranges from reduction of symptoms to complete inhibition of re-infection. In this scenario, it is often difficult to determine the relative importance of sIgA versus serum antibodies, but infection models in knockout mice strongly support the notion that sIgA exerts a decisive role in protection and cross-protection against a variety of infectious agents. Nevertheless, relatively few mucosal vaccines have been approved for human use, and more basic work is needed in vaccine and adjuvant design, including particulate or live-vectored combinations.

Diez et al., 2006 studied the versatility of biodegradable poly(d,l-lactic co-glycolic acid) nano-particulate carrier for plasmid DNA delivery. They optimized different formulations of DNA encapsulated into PLGA particulate carriers by correlating the protocol of preparation and the molecular weight and composition of the polymer, with the main characteristics of these systems in order to design an efficient non-viral gene delivery vector. For that, we prepared poly(D,L-lactic-co-glycolic acid) (PLGA) microparticles with an optimized water–oil–water double emulsion process, by using several types of polymers (RG502, RG503, RG504, RG502H and RG752), and characterized in terms of size, zeta potential, encapsulation efficiency (EE%), morphology, DNA conformation, release kinetics, plasmid integrity and erosion. Concluded that PLGA particulate carriers could be an alternative to the viral vectors

used in gene therapy, given that may be used to deliver genes, protein and other bioactive molecules, either very rapidly or in a controlled manner.

Holmgren et al., 2003 studied on mucosal immunization and adjuvants: a brief overview of recent advances and challenges. Concluded that the development of mucosal vaccines, whether for prevention of infectious diseases or for immunotherapy, requires antigen delivery and adjuvant systems that can efficiently help to present vaccine or immunotherapy antigens to the mucosal immune system. Promising advances have recently been made in the design of more efficient mucosal adjuvants based on detoxified bacterial toxin derivatives or CpG motif-containing DNA, and perhaps even more striking progress has been done in the use of virus-like particles as mucosal delivery systems for vaccines and of cholera toxin B subunit as antigen vector for immunotherapeutic tolerance induction.

Illum, 2003 depicted possibilities, problems and solutions for Nasal Drug Delivery. Depicted problems associated with nasal drug delivery and how it is possible, sometimes by means of quite simple concepts, to improve transport across the nasal membrane. In this way it is feasible to deliver efficiently challenging drugs such as small polar molecules, peptides and proteins and even the large proteins and polysaccharides used in vaccines or DNA plasmids exploited for DNA vaccines. The transport of drugs from the nasal cavity directly to the brain is also described and examples of studies in man, where this has been shown to be feasible, are discussed. Recent results from Phase I/II studies in man with a novel nasal chitosan vaccine delivery system are also described.

Jawahar et al., 2009 prepared PLGA nanoparticles of Carvedilol that will improve the bioavailability of Carvedilol and sustain the release to reduce the initial hypotensive peak and to prolong the antihypertensive effect of the drug. Carvedilol encapsulated by Nano-precipitation method using PLGA and Pluronic F-68.Prepared nanoparticles were examined for physicochemical characteristics, in vitro release kinetics and invivo biodistribution studies. The average size of the nanoparticles were found by them in range of 132-234nm. The drug encapsulation efficiency was 77.6% at 33% drug loading. In vitro cumulative release from the nanoparticles was 72% at 24hr. In vivo

biodistribution studies in rats revealed that these particles are distributed in heart, liver and kidney at higher concentration may allow their delivery to target sites.

Jung et al., 2000 reported the role of polymers in mucosal uptake. Factors such as particle surface charge and hydrophilic/hydrophobic balance of these polymeric materials have not been investigated systematically since adjustment of these particle properties is almost impossible without synthetic modification of the polymers. The current findings will be reviewed and compared to those obtained with nanoparticles consisting of a novel class of charged comb polyesters, poly(2-sulfobutyl-vinyl alcohol)-graft-poly(D,L-lactic-co-glycolic acid), SB-PVAL-g-PLGA, allowing adjustment of physicochemical nanoparticle properties with a single class of polymers.

Kaul et al., 2002 studied long-circulating poly-(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. PEG-modified (PEGylated) gelatin derivative was synthesized for preparation of long-circulating nanoparticles with capacity for intracellular delivery of drugs and genes the nanoparticles in the size range of 200–500 nm were prepared and the presence of PEG chains on the surface was confirmed by ESCA. The results of this study are very encouraging for the development of an intracellular delivery system for drugs and genes that can offer long-circulating property, is efficiently internalized by cells and remains intact in the endosomes and lysosomes.

Li et al., 1997 studied biodegradable system based on gelatin nanoparticles and poly(lactic-co-glycolic acid) microspheres for protein and peptide drug delivery. Their study concluded that the protein containing PLGA-PVA composite may be suitable for long term protein delivery. Also gave an idea about the degradation pattern of the PLGA polymer.

Muthu et al.**,** 2010 reviewed nanoparticles based on PLGA and its co-polymers. This review summarizes the resent studies on PLGA and its copolymers based polymeric nanoparticles. PLGA loaded with different drugs shows effectiveness in their respective therapies. The stealth nanoparticles PLGA co-polymers were developed to overcome the opsonisation process during I.V. administration for an improved and targeted delivery.

Raghuvanshi et al., 2002 described that physical mixture of nano and microparticles resulted in early as well as high antibody titers in experimental animals. Immunization with polymer particles encapsulating stabilized TT elicited anti-TT antibody titers, which persisted for more than 5 months and were higher than those obtained with saline TT. However, antibody responses generated by single point immunization of either particles or physical mixture of particles were lower than the conventional two doses of alum-adsorbed TT.

Rubiana et al., 2004 developed spherical nanoparticulate drug carriers made of poly(d,l-lactide-*co*-glycolide) acid with controlled size were designed. Praziquantel, a hydrophobic molecule, was entrapped into the nanoparticles with theoretical loading varying from 10 to 30% (w/w). This study investigates the effects of some process variables on the size distribution of nanoparticles prepared by emulsion–solvent evaporation method. The results show that sonication time, PLGA and drug amounts, PVA concentration, ratio between aqueous and organic phases, and the method of solvent evaporation have a significant influence on size distribution of the nanoparticles. Release kinetics of PRZ was governed by the initial drug loading, higher initial drug loadings resulting in faster drug release.

Ryan et al., 2001 described immunomodulators and delivery systems for vaccination by mucosal routes. They discussed the immunological principles underlying mucosal vaccine development and review the application of immunomodulatory molecules and delivery systems to the selective enhancement of protective immune responses at mucosal surfaces.

3. RESEARCH ENVISAGED

Immunization efforts against infectious diseases such as polio, smallpox, tetanus and measles have saved innumerable lives by eradicating or decreasing the occurrence of the disease. Despite these impressive results, there is still requirement of novel strategies for the achievement of safe and effective immunization, which are under investigation both in terms of new improved antigens and routes of vaccination. The development of suitable carrier systems remains a major challenge for the pharmaceutical scientists, especially after oral/nasal application/ vaccination since bioavailability is limited by the mucosal barriers of the gastrointestinal tract/nasal route and degradation by digestive enzymes.

Polymeric nanoparticles (NP), defined as solid particles with a size in the range of 10-1000 nm, allow encapsulation of the drugs inside a polymeric matrix, protecting them against enzymatic and hydrolytic degradation. Not only enhancement of nasal peptide bioavailability, but also the concept of mucosal vaccination has become more prominent in recent years.

Neither the hydrophilic nor the hydrophobic system is ideal for protein/peptide drug delivery. They each have their own advantages and disadvantages. For example, the hydrophilic polymeric systems are biocompatible with the protein/peptide drugs, but have difficulty achieving sustained drug release. When the systems absorb water and swell, protein/peptide molecules will rapidly diffuse out. In contrast, the hydrophobic polymeric systems have the capability of yielding sustained drug release. However, they are incompatible with the water soluble protein/peptide drugs. The hydrophobicity of the polymers may induce unfolding of protein/peptide molecules; therefore, the protein/peptide drugs may lose their biological activity after being loaded in and then released from the hydrophobic polymeric systems.

Thus the research work envisaged promotes the advantages and overcomes the disadvantages of both the hydrophilic and the hydrophobic polymeric systems, by a combined hydrophilic-hydrophobic system (gelatin nanoparticles coated with PLGA). This combination can create a new biodegradable system for protein and/or peptide drug delivery. That gives benefit to the society by increasing the effects of proteins/peptide drugs

4.PLAN OF WORK

1. Preformulation studies.
    1.1. Identification and characterization of the antigen.
    1.2. Identification of excipient's.
2. Formulation and optimization.
    2.1. Preparation of gelatin nanoparticles.
    2.2. Coating of nanoparticles by PLGA.
3. Characterization of nano-microsphere composite.
    3.1. Size and size distribution by PCS.
    3.2. Morphology by Electron Microscopy (SEM).
    3.3. Antigen loading efficiency.(TT-FITC Conjugate)
    3.4. Antigen release profile.(TT-FITC Conjugate)
    3.5. Antigen stability (SDS-PAGE).
4. Biological evaluation (Wistar rats)
    4.1. Fluorescence microscopy
    4.2. Immunization protocol
        4.2.1. Primary immunization at 0 Day.
        4.2.2. Booster dose at 14 and 28 day.
        4.2.3. Collection of blood from retro-orbital plexus.
        4.2.4. Estimation of serum IgG level (ELISA).
        4.2.5. Collection of Nasal/Intestinal/Vaginal secretion
        4.2.6. Estimation of sIgA level (ELISA).
5. Compilation of DATA.

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